**Plasmid-reprogramming of human fibroblasts**

Adapted from Okita et al PMID: 21460823

Nucleofection Kit for Amaxa Nucleofector: Normal Human Dermal Fibroblasts – Adult (NHDF-Adult) (VPD-1001)

**Plasmids (from Addgene):**

pCXLE-hOct3/4 (#27076)  
pCXLE-hSK (#27078)  
pCXLE-hUL (#27080)

**Nucleofection (Day 0)- with Amaxa Nucleofector I/II:**

* Prepare nucleofection soloution: 82 µL Human Dermal Fibroblast Nucleofector solution

+ 18 µl Supplement

+ 10 µg of Plasmid

* Nucleofect 700.000 fibroblasts (resuspended in the nucleofection solution) with the P-022 program.
* Distribute nucleofected fibroblasts on a 6-Well plate, coated with Matrigel.
* Culture cells in DMEM + GlutaMAX supplement (Gibco) + 10 % FBS (Gibco) without P/S.

**Day 1:**

* Change medium to DMEM + GlutaMAX supplement + 10% FBS + 2ng/ml FGF2 (Peprotech) with 1 % P/S (Millipore).

**Day 3/4:**

* Change medium to E8 medium + 100 μM Sodium Butyrate (Sigma Aldrich 303410-100G) + 0.1 % P/S and change medium every other day.

**Day 21-28:**

* Pick iPS cell colonies (first colonies should appear around Day 14).